

A08880

TOXICITY OF HALOGENATED HYDROCARBON INSECTICIDES FOR THE FROG, *RANA PIPTENS*

HAROLD M. KAPLAN AND JAMES G. OVERPECK

Current interest in the poisoning of animals by insecticides is widespread. Some studies indicate a virtual eradication of several species in areas that have been treated (Alderdice and Worthington, 1959; Graham, 1960). Occasional reports cite dead or dying frogs, turtles, and snakes (Ferguson, 1963). Gowdey *et al.* (1952) showed that injection of aldrin into frogs produced neurologic dysfunction. Lüdemann and Neumann (1960) demonstrated the toxicity of halogenated hydrocarbons for the larvae of toads. Boyd *et al.* (1963) and Vinson *et al.* (1963) used the frog to determine the possibility of the development of cross-resistance to insecticides.

The present study is concerned with the pathogenicity of several widely used halogenated hydrocarbon insecticides for frogs immersed in such solutions. The toxic doses experimentally found may thus be available for any future comparison with dosages to which frogs may become exposed in natural habitats as fields and ponds or in laboratory aquaria containing water from municipal reservoirs.

MATERIALS AND METHODS

Grossly healthy grass frogs (*Rana pipiens*), all about 3½ inches long and 65 gm. in weight, were isolated several days for manifestations of any latent disease. If no such signs appeared, they were placed at 25°C. in loosely cork-stoppered, one-gallon glass bottles, each containing 200 cc. of a specific concentration of an insecticide in distilled water. There was only one frog per container, and each animal was fed in a separate tank before and during the experimental period. The experiment at each concentration was arbitrarily terminated at 50 days, unless death intervened as it did in higher concentrations. Controls were run in distilled water. All solutions were changed daily and their pH's were taken with a Beckman pH meter.

Seven related halogenated hydrocarbons were used: aldrin, benzene hexachloride (BHC), chlordane, dieldrin, endrin, methoxychlor, and toxaphene.

After 30 days, or else sooner in moribund animals, the frogs were sacrificed. Prior to euthanasia with ether, cardiac and respiratory rates were determined. Total red and white blood cell counts and differential white cell counts were made by routine methods. Neural effects examined throughout the experimental period included activity, posture, tremors, and convulsions. Di-

gestive effects included "salivation" and vomiting. Changes in skin color were noted. Necropsy observations were limited to the size and color of the liver and the presence of hypertemic and petechial spots on the lungs and intestines.

EXPERIMENTAL DATA

All numerical data of frogs adapted to a temperature of 25°C. are summarized in Table 1.

Frogs immersed in the highest concentrations used of aldrin, chlordane, dieldrin, and endrin solutions changed skin color and became grayish. The skin of frogs in BHC changed to a blackish-green. The frogs in methoxychlor and toxaphene showed no distinct color changes. The reasons for these different melanophore reactions are lacking.

The average cardiac and respiratory rates decreased in all frogs in the toxic concentrations and arrhythmias were occasionally produced upon prolonged immersion. The rates were less markedly affected in the more dilute solutions.

Neuromuscular changes were characteristically produced in the toxic concentrations of all the insecticide solutions but were most noticeable in aldrin, chlordane, dieldrin, endrin, and toxaphene. The frogs in these particular solutions thrashed about excessively and became abnormally reactive to stimulation. This behavior persisted. Occasional coarse tremors were observed, and at about 20 days many frogs displayed extensor rigidity. During the fourth week, several frogs had repeated convulsions. Frogs which became moribund always exhibited spastic extensor paralysis, in which condition they made feeble but unsuccessful attempts to right themselves from abnormal postures.

Excessive "salivation" almost always occurred in the spastic animals, often to such an extent that the solutions became frothy. These signs never occurred in the more dilute, nonlethal solutions.

The frogs immersed in the toxic concentrations of BHC were affected differently. They were lethargic and made little or no attempt to escape handling. They maintained the capacity to right themselves. Their hind limbs were flexed and held so close to the body that the profile height of such animals was decreased. Three of 20 frogs held in prolonged immersion in 17 p.p.m. of BHC had exteriorized the stomach by violent vomiting and two others had cloacal prolapse.

Of 20 frogs immersed in toxic concentrations of methoxychlor, five frogs died and these five had exhibited neuromuscular and behavioral patterns similar to those of frogs immersed in the other solutions except BHC. The surviving 15 appeared to be free from any gross neuromuscular disorder.

TABLE 1.—Toxicity of halogenated hydrocarbon insecticides for frogs (*Rana pipiens*) following prolonged immersion.

Insecticide	Conc. of soln. (in p.p.m.)	µl of soln. at 25°C.	No. of frogs used	No. of frogs dead at 30 days*	Blood picture								Avg. heart rate in beats/min. at 30 days*	Avg. change in heart rate at 30 days*	Avg. resp. rate at 30 days*	Avg. change in resp. rate at 30 days*				
					Avg. RBC. in c.mm. at 30 days*	Avg. diff. of RBC. from control at 30 days*	Avg. WBC. no./c.mm. at 30 days*	Avg. diff. of WBC. from control at 30 days*	Avg. differential WBC in per cent at 30 days*											
									Eos.	Baso.	Mono.	Lympho.					Neutro.			
Dist. water control	—	5.05	20	0	471,700	0	18,170	0	4.0	0	8.0	31.0	54.0	75	0	98	0			
Aldrin	0.30	5.90	20	8	303,300	-108,400	14,020	-1,144	14.0	0	10.0	29.0	47.0	58	-17	72	-20			
	0.23	5.71	10	0	430,000	-32,700	14,140	-1,030	12.4	0	3.1	70.2	14.3	62	-13	80	-12			
	0.15	5.08	10	0	532,000	+60,300	14,400	-3,770	19.3	0	0.5	42.1	31.8	72	-3	95	-3			
Benzene hexachloride	17.0	0.23	20	3	275,740	-195,000	12,570	-5,000	5.0	0	10.0	30.0	55.0	52	-23	59	-39			
	8.0	5.85	10	0	400,000	-71,700	12,700	-3,470	13.9	0	7.8	62.5	15.8	60	-15	68	-30			
	0.0	5.82	10	0	430,000	-11,700	13,120	-5,050	0.3	0	7.5	71.4	14.8	62	-13	70	-19			
Chlordane	0.50	5.79	20	8	333,300	-138,400	12,440	-5,730	8.0	0	10.0	29.0	53.0	72	-3	91	-1			
	0.38	5.75	10	0	538,000	+66,300	10,140	-2,030	0.1	0	10.3	74.0	9.0	73	-2	95	-3			
	0.25	5.70	10	0	470,000	+4,300	18,700	+330	20.8	0	4.0	63.8	11.4	73	0	90	-2			
Dieldrin	0.10	5.77	20	10	392,000	-70,700	14,080	-1,000	5.0	0	9.0	33.0	53.0	72	-3	70	-22			
	0.08	5.01	10	0	410,000	-53,700	14,100	-1,010	9.7	1.2	0.2	70.5	12.4	73	-2	94	-1			
	0.05	5.59	10	0	470,000	-1,700	15,190	-2,980	20.2	0.8	10.4	30.2	20.4	75	0	91	-1			
Endrin	0.03	5.80	20	0	312,000	-150,700	13,510	-1,000	4.0	0	10.0	18.0	68.0	55	-20	74	-24			
	0.02	5.80	10	0	438,000	-33,700	15,000	-3,170	18.3	0	2.0	73.4	0.3	61	-14	83	-15			
	0.015	5.78	10	0	452,000	-19,700	10,370	-1,820	11.2	0	2.5	60.3	26.0	69	-0	91	-1			
Methoxychlor	0.80	0.31	20	5	447,400	-21,300	12,900	-3,270	0.0	0	10.0	29.0	55.0	61	-14	71	-27			
	0.60	0.10	10	0	448,000	-23,700	15,920	-2,250	0.2	0	2.7	81.0	10.1	71	-4	91	-7			
	0.40	5.95	10	0	401,250	-10,450	10,070	-1,520	4.5	0	3.7	80.0	0.0	73	-2	95	-3			
Toxaphene	0.00	5.83	20	5	590,500	+124,800	13,200	-4,910	4.0	0	8.0	33.0	55.0	55	-20	70	-28			
	0.45	5.70	10	0	518,000	+40,300	14,150	-4,020	7.0	0	2.0	86.2	3.4	55	-20	85	-13			
	0.30	5.75	10	0	438,000	-33,700	15,190	-2,980	7.8	0	4.3	83.5	5.1	68	-7	94	-1			

* In highest concentrations frogs died at varying intervals of time.

The total erythrocyte count of frogs decreased in the highest concentrations of all solutions used, except toxaphene. In the higher concentrations of toxaphene, the erythrocyte count increased. The total leucocyte count decreased in the highest concentration of all solutions.

The differential white count indicated that the frogs reacted by an increase in the number of eosinophiles. In most cases the eosinophilia was least marked in the most concentrated solutions. The monocytes in many instances showed a slight tendency to rise in the most concentrated solutions, decreasing in the diluted solutions. The neutrophils decreased in the more dilute solutions. The lymphocytes were essentially unchanged in the concentrated solutions except for a marked depression with endrin, but there was always a lymphocytosis in the dilute solutions.

Necropsy observations revealed no gross changes in the lungs of any of the animals. Petechiae occurred occasionally along the intestines of frogs in all the insecticide solutions. The frogs in BHC all had enlarged and hyperemic hearts. The frogs in all other solutions typically had smaller than normal hearts, which in many cases appeared pale and ischemic. The animals in BHC appeared to have enlarged livers, olive to yellowish in color. The animals in all other solutions had livers which were deep blue-black in color.

DISCUSSION

The chlorinated hydrocarbons as a generic group for the most part appear to exert similar effects on the adult grass frog. The most widely used member of the group, DDT, was not included here because there are cognate studies available (Brown, 1951).

The present study is one of chronic toxicity, although the residual actions (i.e., persistence of activity) of the drugs used are not equal. Thus, aldrin evaporates readily and its residual toxicity is shorter than that of the other insecticides used. In general, however, the 30-day period chosen reveals any pathology that is slowly expressed by the test animal.

A comparison of the present data with literature reports indicates that frogs are much more resistant than are fish to poisoning by the halogenated hydrocarbon insecticides. (Gjullin *et al.*, 1949; Henderson *et al.*, 1959; Bennett 1962).

There do not seem to be reports elsewhere indicating pathogenicity of the halogenated insecticides to adult frogs living in natural waters containing ordinary runoff quantities of these drugs. The available information about the known concentrations of the halogenated insecticides in surface waters is fragmentary (Middleton and Lichtenberg, 1960) and suggests wide variations. It therefore can be stated with any certainty from the present data that the

concentrations of halogenated hydrocarbons in natural habitats such as fields and streams are at a pathogenic level for frogs. The probability of increasing danger, however, cannot be dismissed. There is more specific information for aerial sprays, which are stated to have produced injury to frogs (Cottam, 1948; Ferguson, 1963). Actually, the field conditions may be much more severe than those of the present laboratory study, since in the former the insecticides are repetitively introduced whereas in the latter they were introduced at the start and allowed to decay.

In terms of apparent comparative lethality of similar doses upon frogs, the descending order of effectiveness is endrin, dieldrin, aldrin, chlordane, toxaphene, methoxychlor, and BHC. Henderson *et al.* (1959) listed a somewhat similar order in minnows, with endrin most effective and BHC least effective, although Young and Nicholson (1951) considered toxaphene as being the most toxic halogenated insecticide for fish.

The neural signs observed are similar to those reported for fish (Dondoroff *et al.*, 1953), for rabbits used in an immersion study (Johnson *et al.*, 1953), for rats (Lehman, 1949), for chicks (Sherman *et al.*, 1953), and for cattle and sheep (Reco, 1961). The reasons for the dissimilar behavior with BHC are obscure and may be related to the possibility that this compound does not act as an anticholinesterase agent as do the other halogenated insecticides (Cowdrey *et al.*, 1952). BHC did induce gastrointestinal dysfunction since in a few frogs there was vomiting or cloacal prolapse.

The necropsy findings indicate liver involvement. Several reports (Annan *et al.*, 1952; Ambrose *et al.*, 1953) provide evidence that chronic exposure to the halogenated insecticides produces cloudy swelling and centrilobular hepatic cell enlargement.

The heart was pale following immersion in all the insecticides except BHC. Since these compounds are parasympathomimetic (Metcalf, 1955), the vasoconstriction of the coronary vessels that such drugs can produce may explain the bloodless appearance and the variably decreased heart rates following prolonged immersion. The cause of the cardiac arrhythmia in some frogs is obscure.

The respiratory rates of animals of many species which have been poisoned by insecticides are usually elevated (Anonymous Publication, 1952) and the resulting spasms cause death by respiratory failure. Many of the frogs in the present study had passed through prolonged hyperactivity and were eventually severely depressed, the lowered respiratory rates reflecting this condition.

The literature is sparse concerning the hematologic effects of insecticides. DuBois *et al.* (1959) stated that there are no changes in blood counts in man, with the implication that routine blood analysis is of no value in diagnosis. On the contrary, Sanchez-Medial *et al.* (1963) stated that the hematologic effects of the in-

secticides upon man are not sufficiently recognized and that some of these drugs may produce an aplastic anemia. Species differences should also be considered. Nakamura (1960) sprayed rats daily for three months with halogenated hydrocarbon insecticides, following which the animals developed a mild anemia. In the present study with frogs, a similar depression of the total erythrocyte count occurred in the toxic concentrations of all solutions except toxaphene (and a depression was found in the dilute solutions of that drug). The higher concentrations of toxaphene may have stimulated the bone marrow.

The total leucocyte counts were all depressed, except in the most dilute solution of chlordane used. The decreased counts were found to occur concomitantly with marked physical deterioration of the frog and both effects are apparently the results of poisoning. DuBois *et al.* (1959) found no pronounced trend in the differential white cell count in man. No pronounced or consistent trend seems to be evident for the frog, either.

Toxicities are not a result of the moderately acid pH's of the solutions since immersion in distilled water of pH 5.65 produced no symptomatology (Table 1).

SUMMARY

Frogs immersed for long periods in commonly used halogenated hydrocarbon insecticide solutions suffered ill effects in concentrations that in most cases were less than 1 p.p.m. Morbidity or death occurred at or above certain concentrations. The pathologic signs included changes in the color of the skin, digestive upsets, postural defects, lethargy, convulsions, and depression of the total red and white cell counts.

LITERATURE CITED

- ALDERDICE, D. F., AND M. E. WORTHINGTON. 1959. Toxicity of a DDT forest spray to young salmon. *Canadian Fish Culturist*, 24:41-48.
- AMBROSE, A. M., H. E. CHRISTENSEN, AND D. J. ROBINSON. 1953. Toxicology and pharmacological studies on chlordane. *Arch. Indust. Hyg. and Occup. Med.*, 7(3):197-210.
- ANNAU, E., H. KOSST, AND P. J. C. PLUMMER. 1952. Biochemical and histological changes in the liver of mice following feeding of the insecticide aldrin. *Canad. J. Med. Sci.*, 30(6):463-470.
- ANONYMOUS PUBLICATION. 1952. Pharmacological properties of toxaphene, a chlorinated hydrocarbon insecticide. *J. Am. Med. Assoc.*, 149:1135-1139.
- BENNETT, G. W. 1962. Management of artificial lakes and ponds. Reinhold Publishing Corp., New York. iv + 283 pp.
- BOYD, C. E., S. B. VINSON, AND D. E. FERGUSON. 1963. Possible DDT resistance in two species of frogs. *Copeia*, 1963(2):426-429.
- BROWN, A. W. A. 1951. Insect control by chemicals. John Wiley and Sons, Inc., N.Y. vii + 817 pp.

- COTLHAM, C. 1918. Effect of chlorinated hydrocarbons on fish and birds. Address to Am. Assoc. Econ. Ent., New York (December).
- DELPHOS, K. P., AND E. M. K. GEHING. 1959. Textbook of toxicology. Oxford University Press, New York. 302 pp.
- DOUGHERTY, P., M. KATZ, AND C. M. TANZWELL. 1953. Toxicity of some organic insecticides to fish. *Sewage and Indust. Wastes*, 25(7):810-814.
- LARSEN, D. E. 1963. Notes concerning the effects of heptachlor on certain poikilotherms. *Copeia*, 1963(2):141-143.
- GILLIS, C. M., O. B. COPE, B. F. QUENSENBURY, AND F. R. DECHASSON. 1949. The effect of some insecticides on black fly larvae in Alaskan streams. *J. Econ. Entom.* 42(1):100-105.
- GOWDIE, C., A. GRAHAM, J. SEGUM, G. W. SLAVHAKY, AND R. WARD. 1952. A study of the pharmacological properties of the insecticide aldrin (hexachloro-hexahydrodimethanonaphthalene). *Canad. J. Med. Sci.*, 30(4):520-533.
- GRAHAM, R. J. 1960. Effects of forest insect spraying on trout and aquatic insects in some Montana streams. U. S. Public Health Service Technical Report W60-3.
- HENDERSON, C., Q. H. PERKINS, AND C. M. TANZWELL. 1959. The relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. *Trans. Am. Fish. Soc.*, 88(1):23-32.
- JOHNSON, B. L., AND W. G. EDLIS. 1953. The toxicity of aldrin, dieldrin and toxaphene to rabbits by skin absorption. *J. Econ. Entom.*, 46(4):702-703.
- LEHMANN, A. J. 1919. The major toxic reactions of insecticides. *Amer. Prof. Pharmacist*, 15(10):907-911.
- LEHMANN, D., AND H. NEUMANN. 1960. Versuche über die akute toxische Wirkung neuzeitlicher Kontaktinsektizide auf Süßwassertiere. *Z. angew. Zool.*, 47:303-321.
- METCALF, R. L. 1955. Organic insecticides. Interscience Publishers, Inc., New York. iv + 392 pp.
- MURKIN, F. M., AND J. J. LICHTENBERG. 1960. Measurements of organic contaminants in the nation's rivers. *Ind. and Eng. Chem.*, 52:99A-102A.
- NAKAMURO, N. 1960. Experimental studies on chronic poisoning by various kinds of insecticides in rats. *Folia Pharmacol. Japon.*, 56(4):829-849.
- REO, A. 1961. Synthetic halogenated organic insecticides and their toxicology. *Rev. Med. Vet.*, 137(10):761-774.
- SÁNCHEZ-MEDAL, L., J. P. CASTANEDO, AND F. GARCÍA-ROJAS. 1963. Insecticides and aplastic anemia. *New England J. Med.*, 269(25):1365-1367.
- SCHERMAN, M., AND M. M. ROSENBERG. 1953. Acute toxicity of four chlorinated dimethanonaphthalene insecticides to chicks. *J. Econ. Entom.*, 46:1067.
- VINSON, S. B., C. E. BOYD, AND D. E. FERGUSON. 1963. Aldrin toxicity and possible cross-resistance in cricket frogs. *Herpetologica*, 19(2):77-80.
- YOUNG, L. A., AND H. P. NICHOLSON. 1951. Stream pollution resulting from the use of organic insecticides. *Prog. Fish Culturist*, 13(4):193-198.

Department of Physiology, Southern Illinois University,
Carbondale, Illinois.